Microsporidian encephalitis of farmed Atlantic salmon (*Salmo salar*) in British Columbia

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This paper describes the clinical signs and pathology associated with an outbreak of a previously unrecognized microsporidian encephalitis in farmed Atlantic salmon (*Salmo salar*) in British Columbia.

This disease was initially diagnosed in December 1993 at 2 separate netpen sites in the coastal waters of Vancouver Island and continued through January to June 1994. The 1st site, located among a group of islands, contained 217 000 fish (mean weight, 1.7 kg) that had been transferred to saltwater netpens in the winter of 1993. The salmon were distributed evenly amongst 30 cages, each of which measured 15 × 17 m at the surface and extended to a depth of 20 m. Stocking density within the cages averaged 1.4 kg/m^3. The 2nd site, anchored 100 m from a nearby island, was located approximately 3 km away. This site contained 135 000 fish (mean weight, 0.7 kg) that had been transferred to seawater netpens in spring 1993. The salmon were evenly distributed amongst 20 cages, each of which measured 15 m square at the surface and extended to a depth of 20 m. Stocking density within the cages averaged 1 kg/m^3. The following values were representative of water conditions recorded 3 m below the surface at the time of the disease occurrence: temperature 7°C, specific gravity 1.031, and oxygen 6.0 to 6.5 mg/L. The cages at both sites were regularly flushed free of feces and uneaten feed by tidal movement.

Samples of liver and kidney from moribund and fresh dead salmon from both sites were cultured for bacteria on plain tryptone soy agar (TSA) and on TSA with 5% sheep blood. There was no bacterial growth on inoculated agar plates after 10 d aerobic incubation at room temperature. No bacteria were observed in Gram-stained impression smears from liver, kidney, and brain.

Moribund salmon exhibited abnormal swimming behavior, characterized by a slow spiral ascent to the surface with a loss of equilibrium from side to side. Catchable surface "slowswimmers" or moribunds and freshly dead salmon had no consistent external changes, although a few moribund salmon had petechial hemorrhages in the web of the pectoral, pelvic, and caudal fins. Internally, there were no abnormal findings. The stomach was full of feed and there were abundant fat reserves between the pyloric ceca. All fish were weighed, and there was no difference between the mean weights of moribund and healthy salmon. The daily morbidity rate averaged 0.07% for both sites, which was higher than the "acceptable" daily morbidity rate of 0.02%. The daily mortality rate for both sites averaged 0.001%, which was less than the 0.02% budgeted for similarly aged year class. Losses due to predators (seals), early sexual maturation of males, and "non-performers" were the main causes of mortality at both sites.

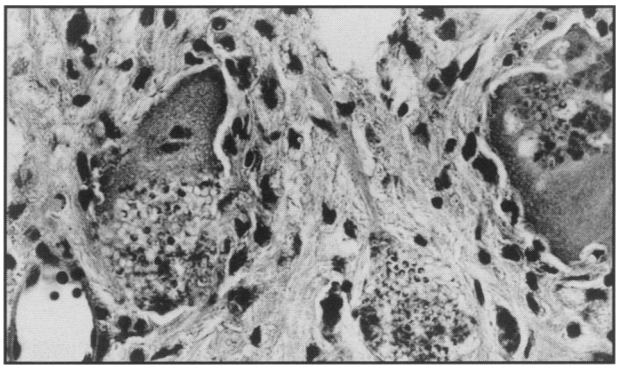

Figure 1. Three motor neurons, in different stages of degeneration, containing large numbers of microsporidian spores. Section of the medulla oblongata of an affected fish. Hematoxylin and eosin. Bar = 30 μm.

Tissues from catchable slowswimmers and fresh dead salmon were fixed in phosphate-buffered formalin (Syndel Laboratories, Vancouver, British Columbia). Microsections were prepared from each fish and stained with hematoxylin and eosin and Gram-twort.

Significant histopathological changes were restricted to the brain of moribund and freshly dead salmon from both netpen sites. The architecture of the myelencephalon was altered along its entire length, due to the presence of degenerating and necrotic neurons and neuronophagic nodules; this was especially dramatic caudally in the medulla oblongata. Typically, degenerate neurons were rounded and had a ground-glass appearance to their cytoplasm, an eccentric nucleus, and in some cases, an increased number of satellite cells. Cells that appeared to be in more advanced stages of degeneration or necrosis had overt cell swelling, karyolysis, and breakdown of the cell membrane. Neuronal necrosis appeared to lead to neuronophagia, in which there was a marked gathering of phagocytic glial cells around neuronal cytoplasmic remnants. Spongiosis of the neuropil was seen, although this was difficult to interpret due to fixation and freezing artifacts in the tissue. The meninges of the brain were normal.

In section, degenerating neuronal cells contained few (3–4) up to massive (>100) numbers of microsporidian spores (Figure 1). These spores were generally present in the cytoplasm of the perikaryon, although in some instances their location extended into the axon. In neuronophagic nodules, spores were present in cytoplasmic remnants of neurons, free in the neuropil, and occasionally in glial cells.

The parasites were identified as microsporidia (phylum Microspora) by the presence of ovoid spores with a posterior vacuole. In sections and fresh wet

Can Vet J 1995; 36: 631–633

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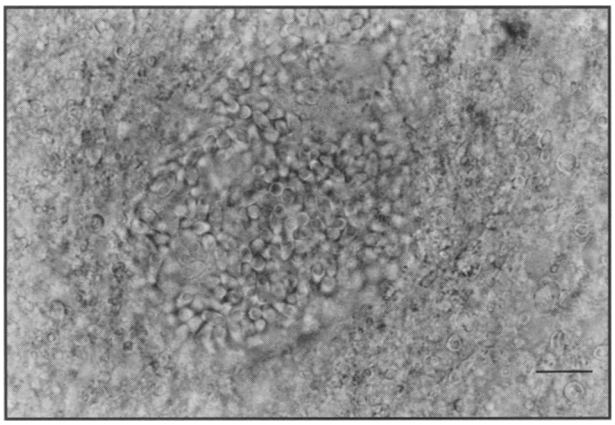

Figure 2. Unstained wet mount from an affected portion of a brain demonstrating an aggregate of ovoid microsporidial spores. Bar = 12 μm.

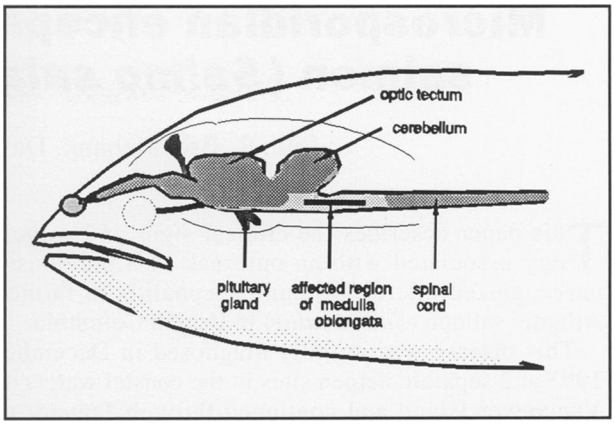

Figure 3. Line drawing depicting regions of the brain of Atlantic salmon (*Salmo salar*) in sagittal section. Infected neurons were most commonly encountered in the highlighted region of the medulla oblongata.

mounts (Figure 2), the spores appeared to be aggregated in groups of either 8 or 16 within a thin-walled sporophorous vesicle, which was not always clearly visible. In fresh wet mounts, individual spores measured 5.7 × 3.0 μm (n = 10). Meronts consisting of about 8 nuclei were rarely detected in neurons. The spores were markedly birefringent; most were strikingly gram-positive and had a sporoplasm that stained intensely blue with both toluidine blue and Giemsa stains.

The microsporidian detected in these 2 separate populations of Atlantic salmon had a general predilection for motor neurons in the myelencephalon, including the paired giant motor neurons (Mauthner's cells) of the medulla oblongata (Figure 3). Mauthner's cells form part of the reticular formation and are bilaterally distributed. These motor neurons are believed to control rapid tail movement (1). Degeneration of these motor neurons, in addition to the effect of an inflammatory response proximate to the surrounding motor tract (fasciculus longitudinalis lateralis) and the nerve root of the vestibulo-acoustic nerve (located just lateral to the Mauthner's cells), may explain the clinical signs observed. Healthy fish were not necropsied; therefore, the presence or absence of microsporidians in them is not known.

The main cause of encephalitis of farmed Atlantic and Pacific salmon in British Columbia is infection with *Renibacterium salmoninarum*, the etiological agent of bacterial kidney disease (BKD). However, this could be ruled out, because, typically, salmon brains infected with this bacterium swim in one direction around the periphery of the seacage. Usually, either the left or the right side of the infected salmon constantly faces the surface of the seacage. Additionally, encephalitis associated with *R. salmoninarum* was not evident on histological examination of these salmon brains.

Microsporidians are common protozoan parasites in the aquatic environment (2), and numerous species are pathogens of fishes (3). Microsporidians are obligate intracellular parasites (2). By contrast with myxosporeans, their spores contain no polar capsules and the polar tube lies free in the sporoplasm (2). Based on light microscopic observations, the precise taxonomic status of the microsporidian herein described could not be determined. Transmission electron microscopy (in progress) is needed in order to determine whether a sporophorous vesicle exists, the actual number of spores within each sporophorous vesicle, and whether the nuclei in the various developmental stages are isolated or diplokaryotic.

Microsporidians are important pathogens of cultured fish (3). Two microsporidians infect seawater-reared salmonids in the Pacific Northwest: *Loma salmonae* infects the gills and, to a lesser extent, the visceral organs of coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*); and *Enterocytozoan salmonis* infects the nuclei of blood-forming cells in chinook salmon (2).

The microsporidian we have described differs from other known microsporidians that infect salmon (i.e., *L. salmonae*, *L. fontinalis*, *Glugea truttae*, and *Microsporidium takedai*) (3). *Loma* spp. form large xenomas, primarily in the gills, and the spores of both species (*L. salmonae* and *L. fontinalis*) are smaller than those of the microsporidian described here. *Microsporidium takedai* (3.4 × 2.0 μm) has been reported only from Japan, and forms cyst-like bodies in muscle tissue. Furthermore, the spores in Atlantic salmon brains are larger than those of *M. takedai*. The microsporidian found in the brain of Atlantic salmon differs from *G. truttae*, which forms a sporophorous vesicle and infects the yolk sac, not nervous tissue, of Brown trout (*Salmo truttae*) in Switzerland. However, the spores of both are similar in size (5.5 to 6.0 μm) and, consequently, further investigation is required to determine whether they are different species.

In addition to differences in hosts and geographic location, the spores from the brains of Atlantic salmon are distinctly larger than those of other microsporidian species infecting nervous tissues of fishes (i.e., *Sprague lophii* (1.4 × 3.7 μm) from *Lophius* spp., *Pleistophora* spp. (2.5–3 × 1.5–2 μm) from *Fundulus heteroclitus*, and *Microsporidium* spp. (1.5–2 × 3–4 μm) from *Brachydanio rerio* and *Trachurus declivis*) (3).

No other microsporidians have been described from nervous tissue of fishes in the Pacific Northwest. The genus *Microsporidium* is used to identify an

assemblage of identifiable microsporidian species, in which insufficient data are available to assign these organisms to specific genera (4). The microsporidian from the brains of Atlantic salmon is clearly an undescribed species, but the essential information on its development that is needed for determining its precise generic status is not available. We, therefore, propose the name *Microsporidium cerebralis* for this organism.

The absence of a weight difference between moribund and healthy salmon indicated that microsporidian-infected salmon were capable of reaching harvestable size during the same growout period as healthy salmon.

Currently, there is no approved chemotherapeutic agent in Canada for the treatment of microsporidian diseases of farmed fish.

Under experimental conditions, fumagillin (Fumagillin-B, Medivet DCH, Sanofi Animal Pharmaceuticals Ltd., High River, Alberta), approved for the control of microsporidiosis (*Nosema* spp.) in apiaries, has been used successfully to treat *E. salmonis* in chinook salmon, administered PO at 1.0 mg activity/kg body weight of fish for 14 d consecutively (2), and *L. salmonae* in seawater-reared chinook salmon at 10 mg active/kg fish for 30 d consecutively (5).

To prevent entrapment and subsequent death of microsporidian-infected salmon in seacage pockets, created by tidal movement, additional downhauls (25 kg) were placed in the corners and along the sides of the seacages. CVJ

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